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14. ABSTRACT The aim of this proposal is to investigate the role of Ezh2 in prostate cancer progression. Ezh2 is a histone methyl transferase whose activity requires to be part of a multi-protein complex. We have hypothesized that the complex composition would change during the prostate tumorigenic progression. To address this question, we have generated a knock-in mouse where Ezh2 is tagged to allow the immunopurification of its associated polypeptides from limited amount of starting material (mouse prostate). The knock-in mice are being generated. Using the targeted ES cells, I am currently setting up the micropurification protocol. One of the Ezh2 associated protein is Eed, different isoforms of this protein are detected <i>in vivo</i> . These isoforms are supposed to be key determinants of Ezh2 substrate specificity. I have characterized these isoforms and generated tools allowing the overexpression of each isoforms independently.					
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Introduction

The aim of this proposal is to investigate the role of Ezh2, a histone lysine methyltransferase, in prostate cancer progression. Ezh2 is overexpressed in several types of cancer including prostate cancer however whether Ezh2 up-regulation is a consequence of or a cause of cancer progression is unknown. Ezh2 is a key regulator of the epigenetic code that is involved in establishing and maintaining cellular identity. The methyl modifications of histone tails are believed to be irreversible. Many studies focus on which enzymes are responsible for the methyl marks, which histones are the targets of each enzyme and how this irreversible mark gets transferred to new DNA during replication. Changes in the histone lysine methyl marks are expected to have profound impacts on chromatin structure and therefore on gene expression.

Body

Task 1. PRC components expression and specific histone methylation during prostate cancer progression in tissues from mouse models

I have analyzed the expression of the PRC components at different stages of prostate cancer progression and observed that in addition to Ezh2, Eed, Suz12 and Sirt1 are also over-expressed in advance stages of prostate cancer. Analyses of the methylation status of histone H1 appear to be more complicated than initially anticipated as our 3meH1K26 antibody characterized using purified proteins cross react with the histone H3. I am working on lowering the cross reactivity by competition with H3 peptide.

Task 2. Biochemical purification of PRC complexes as a function of tumor stage

The main focus of my work, since October 2005 when the grant started, has been to generate knock-in mice in which Ezh2 is tagged by a FLAG and SBP recognition sequence. I have designed and generated the construct to target the gene and selected homologous recombinant ES cells. These cells have been injected, chimera mice are obtained. Germline transmission is being checked.

Using the targeted ES cells, pilot experiments are being performed to set up the micropurification protocol.

Task 3. Modulation of PRC component expression in tissues and testing for cancer progression

As stated in the proposal, we plan on modulating the expression of each PRC component with additional focus on the Eed isoforms. Work done for another study revealed that our initial hypothesis, based on a previous publication, regarding the translation start site of each Eed isoform was inexact. Using 2 dimensional gels followed by mass spectrometry, I have re-characterized these Eed isoforms. Based on these results, I have generated mammalian expression plasmids for each of the isoforms. My aim is to infect prostate tissue with each isoform in order to investigate their individual as well as combined roles in prostate cancer progression.

Key research accomplishments

- 1) the Ezh2 knock-in mouse are being generated.
- 2) the eed isoforms have been further characterized and plasmid allowing expression of each independent isoforms generated.

Reportable outcomes

None so far.

Conclusion

In the past year, a growing number of publications have described the overexpression of Ezh2 in various types of cancer including prostate cancer. Furthermore, it's now well admitted that epigenetic plays a crucial role in the aberrant pattern of gene expression associated to tumorigenic initiation/progression. However the importance of Ezh2 in mediating the epigenetic changes associated to the prostate cancer progression are still elusive. I have generated valuable tools to understand how the upregulation of Ezh2 might lead to gene dysregulation.

Appendices

In September 2006, Dr Danny Reinberg's laboratory moved to New York University. This move doesn't affect the achievement of this proposal as all the facilities available at the University of Medicine and Dentistry of New Jersey exist also at New York University. Furthermore, all the collaborations (Drs Cory Abate-Shen and Michael Shen) are still effective. However, I am facing some delays in the mouse work due to time required to transfer animals from one vivarium to the other.